further 3.3% over the subsequent second to fifth load cycles (the permanent deformation decreasing in percentage terms with each subsequent cycle).

[0256] Conclusion:—

[0257] This data demonstrates the resilience of the material as measured by the low percentage irrecoverable stress deformation in subsequent cycles after the first load cycle.

EXAMPLE 7

Protocol for Testing the Fibroin Material: Resilience Testing B

[0258] Method:—

[0259] The Mechano-activated tissue engineering (MATE) system was used to analyse six samples of optimised fibroin material measuring 8 mm×4 mm.

[0260] The samples were not cross-linked and had an average pore size of 200 μm. Neither were the samples seeded with cells and the mechanical behaviour only of the empty samples was examined.

[0261] The samples had been dehydrated for storage and were rehydrated in Phosphate Buffered Saline (PBS) without problem by centrifugation in the medium.

[0262] Rehydrated samples were analysed over a period of 5 days using a loading regimen as follows: cycle rate: 3 Hz, force amplitude: 5 N minus a preload of 0.2 N, temperature: 37°, medium, PBS.

[0263] Results:—

[0264] After 1,200,000 cycles no indication of material degradation was observed and the material of the samples appeared structurally similar to the beginning of the experiment. Measurement of the samples revealed that the material thickness had decreased on average by 8.2% (from 3.88 mm to 3.57 mm)

EXAMPLE 8

Protocol for Testing the Regenerated Silk Fibroin Solution: Gelation Time

[0265] Method:—

[0266] Drops of regenerated silk fibroin solution with a concentration of about 8% w/v were placed in the bottom of a plastic petridish and were exposed to vapour at room temperature from a drop of glacial acetic acid placed on a filter paper in the lid of the dish. To establish the gelation time the drop was probed with a plastic Eppendorf pipette tip. The material was deemed to have gelled when it would no longer flow when the dish was tilted or when probing the surface of the drop produced an irrecoverable deformation in the surface of the drop.

[0267] To compare the gelation time of the optimised regenerated fibroin solution directly with the fibroin solution disclosed in US2007/0187862, the protocol described therein was replicated.

[0268] The pH of the optimised regenerated fibroin solution containing 8% w/v fibroin was adjusted to pH 6.5-6.8 using very dilute hydrochloric acid or sodium hydroxide solutions and the concentration determined by refractometry using a calibration curve prepared from over 100 samples of optimised dialysed fibroin solution whose fibroin concentration had been determined by gravimetry. 0.5 ml aliquots of this solution were transferred to small cylindrical glass tubes with an internal diameter of 10 mm which were sealed with parafilm. The tubes were incubated either 60° C. and inspected regularly. The time taken for the material to cease to flow when the tubes were inverted was determined.

[0269] FIG. **7** in US2007/0187862 gives an average gelation time of 4 days at 60° C.

[0270] Results:—

[0271] The gelling time for the optimised silk fibroin measured in this way was 5 hours. Thus the gelation time for the optimised regenerated fibroin solution reported herein is approximately 20 times faster than for the silk fibroin solution reported in US2007/0187862. This taken together with the evidence from rheological testing described demonstrates the superiority of the optimised regenerated fibroin solution over that disclosed in US2007/0187862.

EXAMPLE 9

Protocol for Testing the Regenerated Silk Fibroin Solution: Liquid Crystallinity

[0272] Method:—

[0273] To investigate whether the regenerated silk fibroin solution could form a liquid crystalline mesophase, droplets of the approximately 8% w/v optimised regenerated fibroin solution were placed on glass slides with or without coverslips and with or without adjustment to pH 6.5 with 0.1 M ammonium acetate buffer. Slides were allowed to dry slowly at 4° C. by placing the slide in a plastic Petri dish with a lid. Under these conditions small spherulitic crystals of fibroin slowly formed in the fibroin.

[0274] Results:—

[0275] As shown in FIG. **2**, when examined under the polarizing microscope most of the samples showed a maltese cross pattern with four radial isogyres. The liquid phase surrounding the spherulites showed a pattern of irregularly curved isogyres, some of which are continuous at their origin with the isogyres of the spherulites indicating a calamitic liquid crystalline phase. The largest spherulite in the micrograph has a diameter of 10 μm.

[0276] This indicates that, like native silk fibroin in, or taken from, the silkworms silk gland, the optimised regenerated silk fibroin solution is capable of forming a calamitic liquid crystalline mesophase. This effect is not seen in regenerated silk solutions prepared by the standard protocol described in the literature. These observations demonstrate that the optimised regenerated silk fibroin prepared according to the protocol of the invention closely resembles native silk fibroin taken directly from the silk gland in the animal and is superior to that prepared by the conventional process described in the literature. It will be appreciated that the ability to form a mesophase is important for allowing the fibroin molecules to be readily orientated in the pore walls during the freezing step.

EXAMPLE 10

Protocol for Testing the Regenerated Silk Fibroin Solution: Rheological Testing

[0277] Rheometry was used to investigate whether samples of the optimised regenerated silk fibroin solution had rheological properties close to that of native silk fibroin and very different from that of regenerated silk fibroin prepared by the standard protocol disclosed in the literature. The protocol for investigating the rheology of silk fibroin solutions is described below.